



Bighorn Sheep and Mountain Goat Herd Health Assessments

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Project Background:

Respiratory disease continues to have pronounced effects on BHS populations throughout the western US (Aune et al. 1998, Gross et al. 2000, Singer et al. 2000, Cassirer et al. 2007, Cassirer et al. 2013). In Montana, bighorn sheep (BHS) populations have experienced approximately 25 known respiratory disease epizootic events since 1979 (Sells et al. 2015). During the single winter of 2009-10, 4 BHS populations covering a large portion of western Montana experienced simultaneous all-age die off events due to respiratory disease (Edwards et al. 2010).

Translocation is a commonly used tool for the management of BHS in Montana and has historically been used in mountain goat (MG) population management as well. The need for proactive health monitoring to guide translocation decisions is well understood in Montana (Carlsen & Erickson 2010). Research suggests that translocated BHS face higher mortality rates from pneumonia when compared to their resident counterparts (Plowright et al 2013). In addition, there are now several observed cases throughout the western US of pneumonia die-offs in recipient BHS herds following a translocation event, at least raising the possibility that new pathogens or strains were inadvertently introduced or that immunologically naïve transplants fueled local disease transmission. Respiratory disease, and its associated pathogens, serves as one important example of a larger range of infectious organisms that are capable of affecting translocation success in both BHS and MG, and for which proactive monitoring prior to translocation could improve the success of these actions. Montana Fish, Wildlife, and Parks' (FWP) 2010 Bighorn Sheep Conservation strategy states that Montana will obtain and utilize health profiles for both donor and recipient herds to help guide BHS translocations (Carlsen & Erickson 2010).

Mountain goats and BHS are susceptible to many of the same parasites and pathogens and often overlap in distribution, raising the question of how these two species may affect each other's health, including the translocation of one species into another species' range. An outbreak of respiratory disease among sympatric MG and BHS in Nevada in 2013 underscores the potential for these two species to be sharing parasites and pathogens (P. Wolff, Nevada Department of Wildlife, unpublished data). Mountain goats in the Greater Yellowstone Area share the same respiratory pathogen community with BHS populations in the region, indicating the potential for cross-species transmission in Montana (Lowrey et al. 2018).

This report covers the third year of what is intended to be a long-term effort to evaluate the pathogen communities and baseline health status of BHS and MG herds across Montana. This information on BHS and MG herds is needed to inform translocation efforts that are intended to help conserve these species, and to minimize the introduction and spread of novel pathogens within and between the two species. As part of FWP's Bighorn Sheep and Mountain Goat Health Program, we have developed a sampling and monitoring plan consistent with programs in other western states and which prioritizes pathogen sampling for herds that are likely to be involved in translocation events in the near future, either as donor or recipient herds.

Methods:

Study areas

In 2017, FWP regional wildlife program managers identified the Spanish Peaks BHS herd (HD 301) and the Gallatin Crest MG herd (HD 314) as being of interest for sampling to inform current management and for near-future use as translocation source herds. In addition, 20 BHS were translocated out of the Middle Missouri Breaks herd (HD 622) and released in the Clark Fork Cut-off herd (HD 122) in Region 1 to increase population size. We sampled BHS in HDs 301 and 622 and MG in HD 314 for baseline health information (Table 1).

Table 1. Target and actual sample sizes, for winter 2017-2018 sampling of bighorn sheep. Numbers of captured yearlings and lambs, and adult (≥ 2 yrs) females and males are provided.

					Information from handled animals				
HD & Herd name	Est. herd size	Recent (5 yr) demographic performance	Population objective	Target sample size	Date of capture	Total animals caught	Adult Females (≥ 2 yrs)	Adult Males (≥ 2 yrs)	Lambs/Kids & Yearlings
BHS HD 301: Spanish Peaks	172	Stable	150	30	2/20/2018	15	13	2	0
MG HD 314: Gallatin Crest	268	Increasing	NA	30	2/12/2018	19	9	8	2
BHS HD 622: Middle Missouri Breaks	236	Stable	175-200	20	2/10/2018	20	14	0	6

BHS HD 301: Spanish Peaks

This population is a native sheep herd that has been augmented with 10 rams spread across 3 separate occasions, in 1944, 1947, and 1963. The population was generally stable between 75-150 bighorn counted for many years. This herd has not experienced an all-age die-off, but it has experienced successive years of poor lamb recruitment. A recent period of poor lamb recruitment in the early 2000's resulted in as few as 50 BHS detected during aerial surveys and a temporary closure of the area to hunting. During this time, a special mountain lion management area was implemented on top of the bighorn winter range to discourage mountain lion predation on the struggling herd. By 2010, more than 200 BHS were observed on the winter range, a record high count. Unfortunately, this high count was followed by a severe winter and the starvation loss of many sheep. As the population rebounded, the first ever ewe hunt was implemented to maintain sheep counts below 200 to prevent further starvation loss incidents. Concurrently with the highest counts ever observed on the main winter range, we have noted new dispersal events. Up to 20 BHS have been seen in Jack Creek in winter time, approximately 20 miles from their traditional winter range. The sporadic observations of BHS in Beartrap Canyon have continued summer through winter, and are becoming more predictable, with up to 12 BHS observed there, approximately 30 miles from traditional winter range. In 2018, MFWP documented the first known interchange between the Spanish Peaks herd and the Taylor-Hilgard herd to the south. The Taylor-Hilgard herd core winter range is approximately 35 airline miles away from the Spanish Peaks herd. An ear-tagged ewe transplanted to Wolf Creek as part of the within-mountain range

augmentation effort there was observed along Highway 191 two years later. Connection between these herds is expected to strengthen in future years.

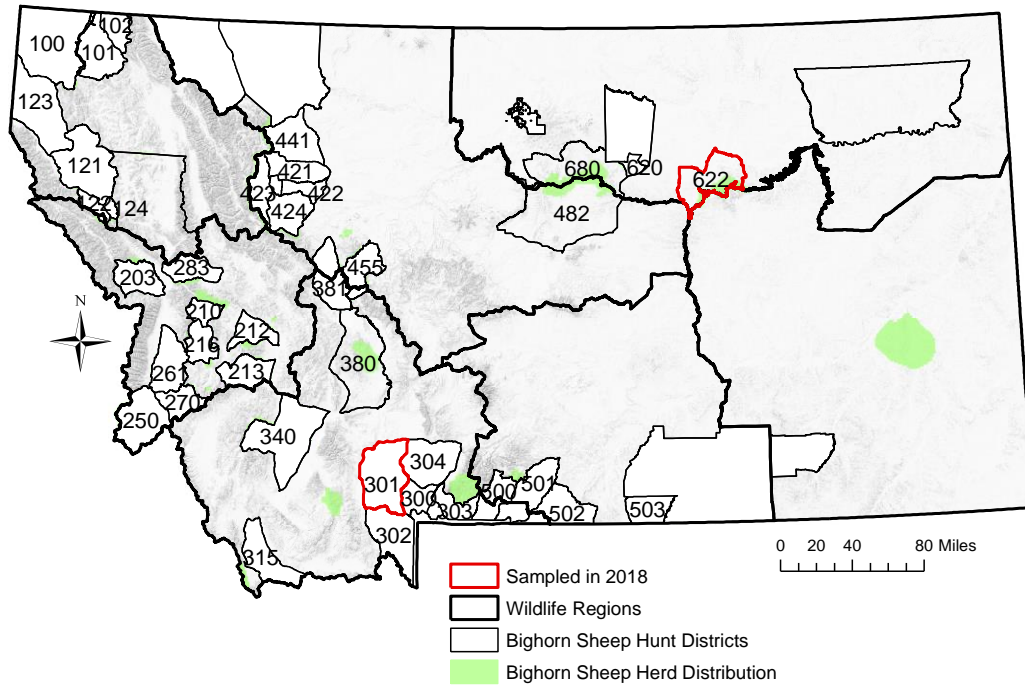
MG HD 314: Gallatin Crest

Mountain goats were first detected on the Gallatin Crest during a bighorn sheep survey in 1992 and were thought to have naturally colonized from non-native populations in either the Absaroka Range or the Spanish Peaks. The first dedicated mountain goat survey was conducted in 1997 and resulted in a total of 38 goats in the southern portion of the range. Goats gradually expanded their range into the north end of the district and currently inhabit all suitable habitat along the Gallatin Crest. The most recent count in August 2017 resulted in 238 goats, which is the highest number ever observed. A hunting season was opened for goats in 1996 with a quota of 2 licenses. As the population has expanded hunting opportunity has been increased with the license quota currently set at 30. Mountain goats share the Gallatin Crest with several herds of bighorn sheep which summer in the Hyalite area, the Ramshorn area and along the Yellowstone Park boundary. There have been two disease outbreaks among bighorn sheep on the Gallatin Crest, including a pneumonia event among sheep that summer in the Hyalite area in 2013, and a second pneumonia event among sheep that summer in the Ramshorn and Park boundary areas in 2015. In the fall of 2016 a hunter observed a dying mountain goat in the Hyalite area. FWP staff were able to locate and collect samples from the dead goat, resulting in confirmed respiratory disease. During the August 2017 survey, observed kid ratios were at or above average in the middle and southern portions of the district, and below average in the Hyalite area. Despite this, it does not appear that disease is having population level impacts as the population has continued to increase. Likewise, the sympatric bighorn sheep population has been increasing in recent years with no recent indications of active disease.

BHS HD 622: Middle Missouri Breaks

The Middle Missouri Breaks BHS herd is located north of Fort Peck Reservoir (Figure 1). This herd was founded in 1980 through a reintroduction effort using 28 BHS from the Gibson Lake North (HD 423) herd. The overall population has steadily increased from the original 28 reintroduced animals to over 300 BHS (Figure 2). This population was historically comprised of two subgroups, the smallest one located in the Mickey and Brandon Buttes area and the larger subpopulation in the Iron Stake/Larb Hills area and has recently expanded east of Timber Creek to the Bone Trail area. The smaller of the two subpopulations averaged 50 BHS between 1986 and 2010, peaked at 80 BHS in the mid-1990s, and has decreased annually over the last 10 years until six sheep were observed during 2016 surveys and none have been seen in that area since. The larger of the two subpopulations has increased since 2010 from 160 to a high of 318 BHS in 2016. This herd was used as a source herd for a translocation of 20 BHS to the Cut-off herd in HD 122 in 2018.

A



B

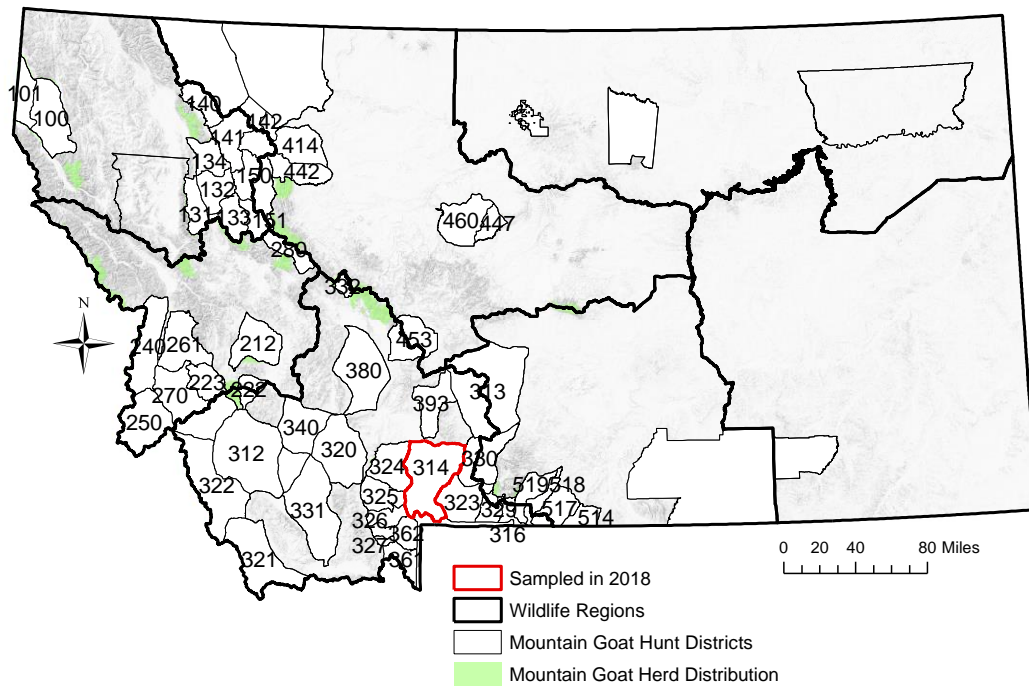


Figure 1. Map of (A) Montana's BHS and (B) MG hunt districts (outlined and numbered) and associated herd distributions (green polygons). Herds within hunt districts outlined in red were visited for health sampling in 2018.

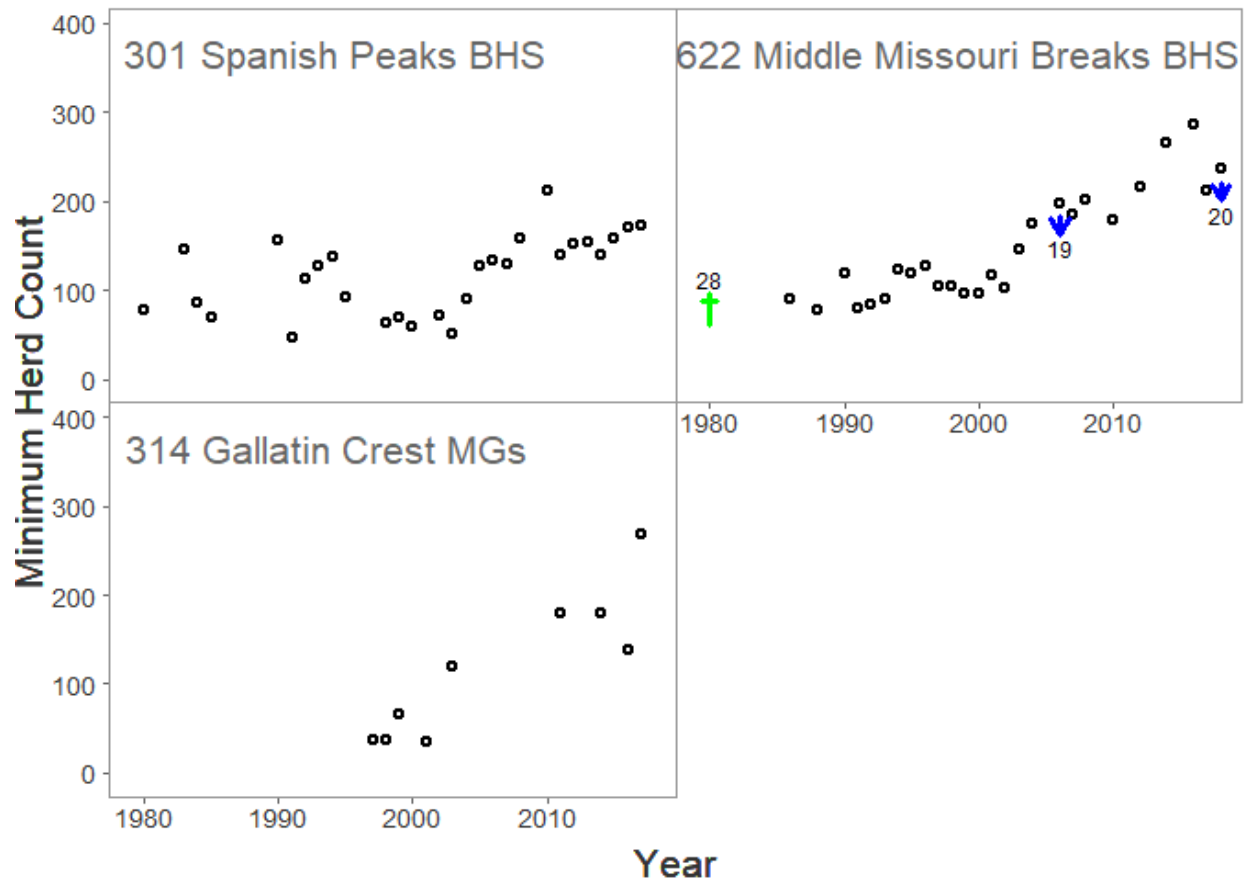


Figure 2. Minimum herd counts over time for the sampled BHS (HD 301 and 622) and MG (HD 314) herds. There have been no observed all-age pneumonia die-offs within any of these herds. Historic translocations into and out of the herds are denoted with green plus and blue arrow symbols, respectively, with the number of animals moved noted. Surveys of the Spanish Peaks' BHS are conducted by helicopter between March-May, Middle Missouri Breaks BHS are typically conducted biennially by helicopter in February, and Gallatin Crest MGs by fixed wing (pre-2011) or helicopter (post-2011) in July-August (except in 2014 & 2016, when they were conducted between February-March).

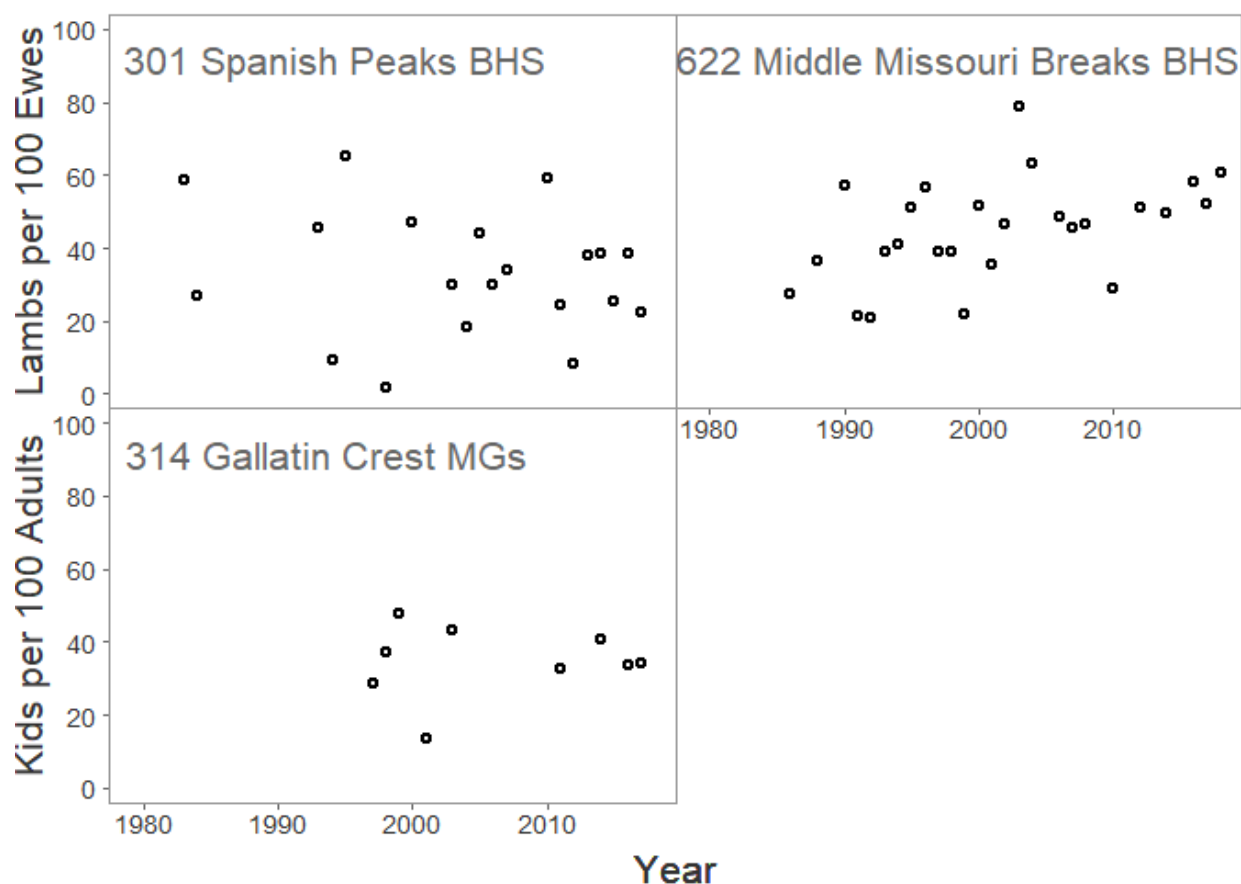


Figure 3. Minimum lambs per 100 ewes and kids per 100 adults observed over time for the sampled BHS (HD 301 and 622) and MG (HD 314) herds, respectively. Surveys of the Spanish Peaks' BHS are conducted by helicopter between March-May, Middle Missouri Breaks BHS are typically conducted biennially by helicopter in February, and Gallatin Crest MGs by fixed wing (pre-2011) or helicopter (post-2011) in July-August (except in 2014 & 2016, when they were conducted between February-March).

Data collection

Sample sizes, animal capture and handling

All capture and handling procedures for sheep and goats were conducted according to the Montana Fish, Wildlife, and Parks Bighorn Sheep Biomedical Protocol (Montana Fish, Wildlife and Parks IACUC #FWP03-2018). In February 2018, Montana Fish Wildlife and Parks staff and Quicksilver Air captured 15 Spanish Peaks BHS, 19 Gallatin Crest MGs, and 20 Middle Missouri Breaks BHS via helicopter net gunning (Table 1). Target sample sizes for each population were defined to detect respiratory pathogens with at least 95% confidence, assuming pathogen prevalence was 10% at the population level. Work by Butler et al. (2017) showed that detection probabilities based on culture for many respiratory pathogens of interest are lower than previously thought. To account for imperfect pathogen detection, we collected and tested duplicate tonsil swabs to increase our confidence in pathogen detection.

Efforts were made to broadly sample individuals within and across all known subgroups of each herd. We preferentially targeted adult female BHS and MGs for capture and sampling. Captured animals were hobbled and blindfolded by the capture crew and either processed on site or transported to a central processing base. Upon restraint, we monitored vital signs while processing each animal. Captured animals were fitted with red plastic ear-tags prior to release. In addition, GPS-collars were deployed on 13 BHS captured in the Spanish Peaks herd (HD 301) to obtain basic home range and movement information.

The Spanish Peaks' BHS herd inhabits the Lee Metcalf Wilderness Area and the Gallatin Crest MG herd inhabits the Gallatin Crest Wilderness Study Area. FWP worked closely with the USFS to obtain permits to capture within both of these areas.

Sample Collection

Sheep and goats captured as part of the Bighorn Sheep and Mountain Goat Herd Health Assessment Project received a full health inspection and evaluation including the collection of information on age, sex, a genetic sample, whole blood, blood serum, fecal samples, nasal and tonsil swabs (multiple tonsil swabs were collected and tested for all animals; swabs were placed in Tryptic soy broth medium and frozen at -80C until tested), and a sampling of any external parasites. Body condition scores were collected where possible. A variety of assays were employed to detect (1) a range of parasites and pathogens known to be relevant to BHS health and management (Appendix I; Carlsen & Erikson 2010), (2) trace minerals, and (3) physiological condition, including pregnancy status and a body condition score for BHS on a scale of 0.5-6 following a protocol developed by Tom Stephenson (personal communication). Samples were collected and data were analyzed according to standard protocols (Western Association of Fish and Wildlife Agencies 2015). Extra blood serum, swabs, and genetic samples (gene cards or biopsy punches) were collected and archived for future testing and analyses.

Animal and Field Site Monitoring

Aerial surveys were used to monitor population trend and recruitment ratios in each sampled herd. The area wildlife biologist assigned to each herd conducted aerial surveys (surveys of the Spanish Peaks' BHS are conducted by helicopter between March-May, Middle Missouri Breaks BHS are typically conducted biennially by helicopter in February, and Gallatin Crest MGs by fixed wing (pre-2011) or helicopter (post-2011) in July-August (except in 2014 & 2016, when they were conducted between February-March)).

Lab analyses

All BHS and MG pathogen and parasite testing was carried out in accordance with standard protocols from the Western Association of Fish and Wildlife Agencies (Western Association of Fish and Wildlife Agencies 2015; Appendix I). Leukotoxin A PCR testing was conducted from a swab of the bacterial growth from the primary streak zone of the culture plate. Blood trace mineral levels and fecal parasitology were performed at Michigan State University's Diagnostic Center for Population and Animal Health. Pregnancy-Specific Protein B assays were run at BioTracking Inc. to determine pregnancy.

Data analyses

For each herd, we estimated the proportion of the herd exposed (for serology tests) or infected (for PCR or other direct tests) with each pathogen, mean blood trace mineral levels, and mean body condition indices. Using estimated detection probabilities for respiratory pathogens from Butler et al. (2017), we corrected raw estimates of exposure and infection rates by dividing the raw estimates by estimated detection probabilities and calculated corrected confidence intervals using the delta method. When we failed to detect the presence of a respiratory pathogen, we calculated our statistical power to detect the pathogen if it were present, using the approach detailed in Butler (2017). Statistical power is defined here as the probability (ranging from 0-1) that we would have detected the pathogen if it was present in the herd at 10% prevalence, given the herd size, our sample size, number of swabs collected per animal, and the estimated detection probability. Point estimates and confidence intervals for proportion and probability statistics were estimated with the binomial distribution. Point estimates and confidence intervals for continuous statistics were estimated with the normal distribution.

Results

BHS HD 301: Spanish Peaks Bighorn Sheep

On February 20, 2018, FWP and Quicksilver Air captured 15 BHS in the Spanish Peaks herd, including 13 adult females and 2 adult males. Within this herd, we found evidence of exposure to, and infection with *Mycoplasma ovipneumoniae*: 53% (95%CI: 30-75%) were seropositive, and 7% (95%CI: 10-30%) tested positive by PCR (Figure 4 & Figure 5). We detected the presence of *Mannheimia haemolytica* and found that 13% (95%CI: 4-38%) of animals tested positive for the leukotoxin A gene from *Pasteurellaceae* cultured from tonsil swabs. While we failed to detect *Pasteurella multocida*, additional hemolytic *Mannheimia* species or hemolytic *Bibersteinia trehalosi*, we lacked sufficient power to do so (Figure 5). We did detect non-hemolytic *Mannheimia* species and *Bibersteinia trehalosi* (Figure 4). In addition, we detected exposure to several moderate risk pathogens (Appendix I), including contagious ecthyma, anaplasma, epizootic hemorrhagic disease virus, *Leptospira sp.*, and parainfluenza type-3, and detected active infections with *Protostrongylus* lungworm species (Figure 6; Table 2).

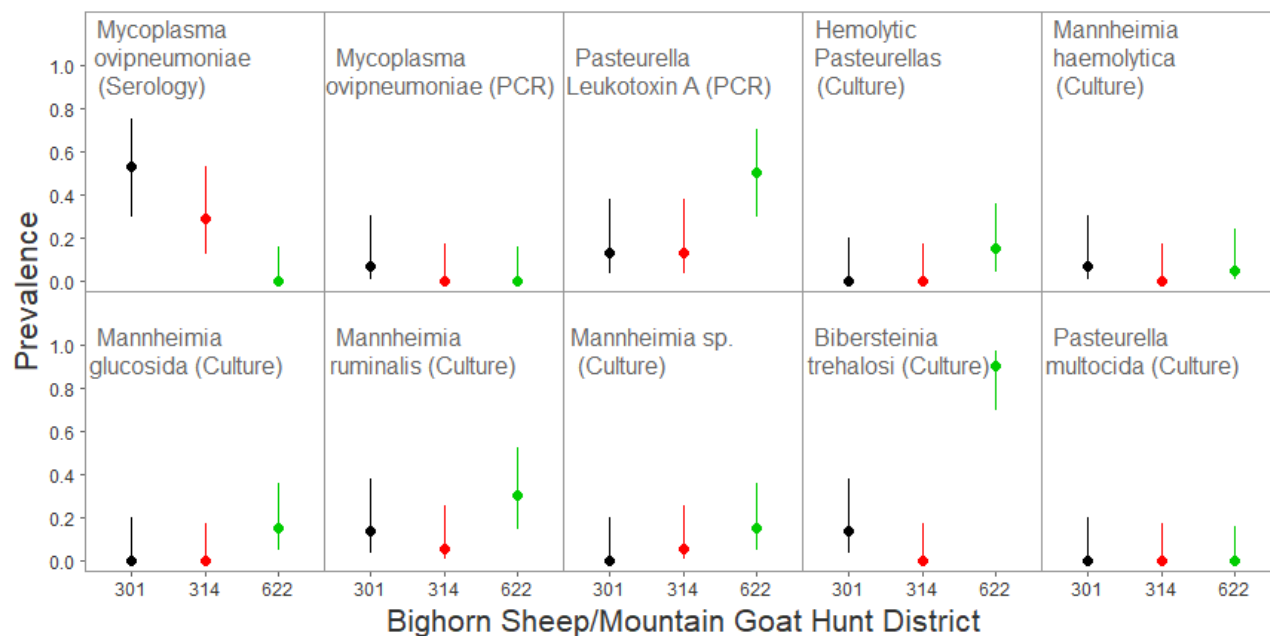


Figure 4. Summary of raw prevalence (proportion testing positive) and associated 95% binomial confidence intervals for respiratory pathogen exposures by herd. Data includes information from serology, PCR, and culture tests, as noted. This figure does not account for imperfect pathogen detection (see Figure 5).

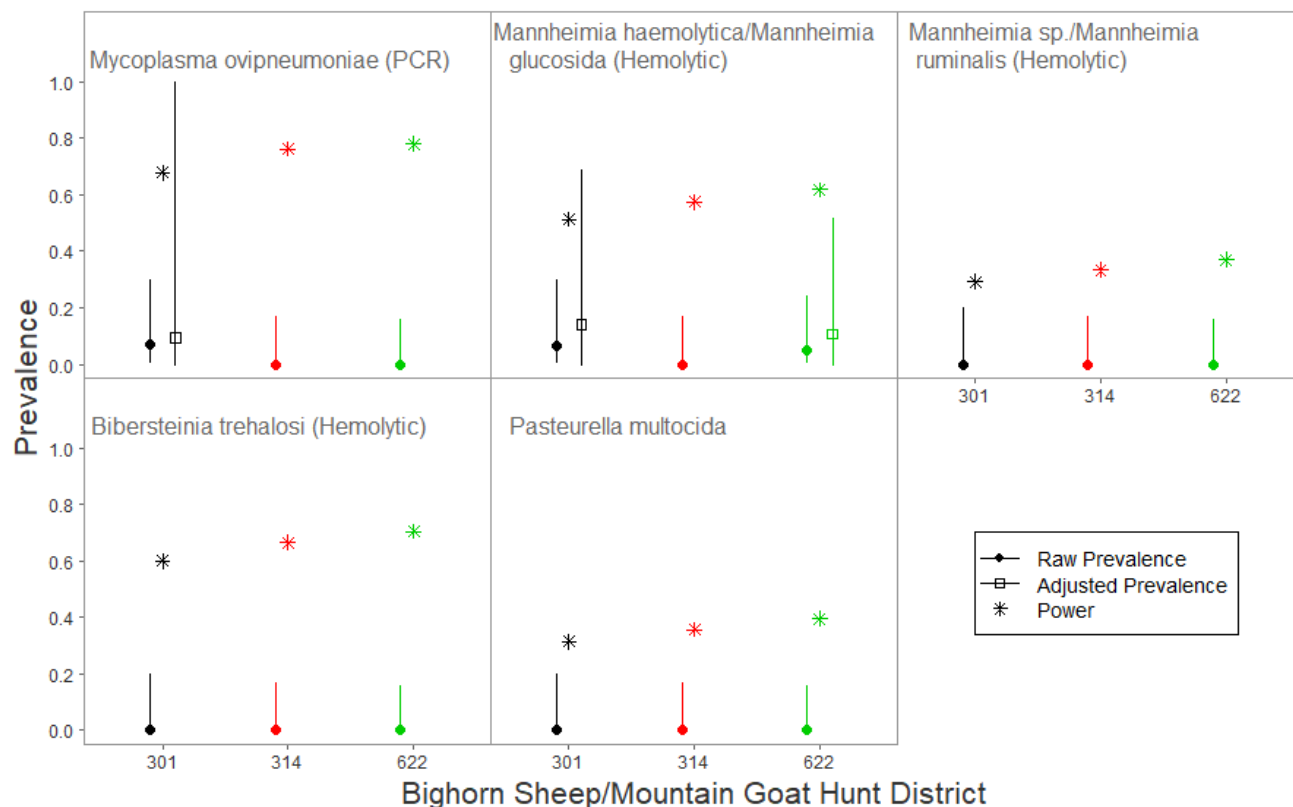


Figure 5. Summary of estimated raw and adjusted (for imperfect detection) prevalences, and associated 95% confidence intervals, as well as estimated power to detect select respiratory pathogens. Adjusted prevalences were calculated by dividing the raw estimates by estimated detection probabilities from Butler (2017), and corrected 95% confidence intervals were calculated using the delta method. We estimated the statistical power to detect the pathogen (denoted by the asterisk symbol) if it were present at 10% prevalence given the herd size, our sample size, number of swabs collected per animal, and the detection probability using the approach detailed in Butler (2017). Point estimates and 95% confidence intervals for raw prevalences were estimated using the binomial distribution. Cultured *Mannheimia haemolytica* was assumed to be beta-hemolytic.

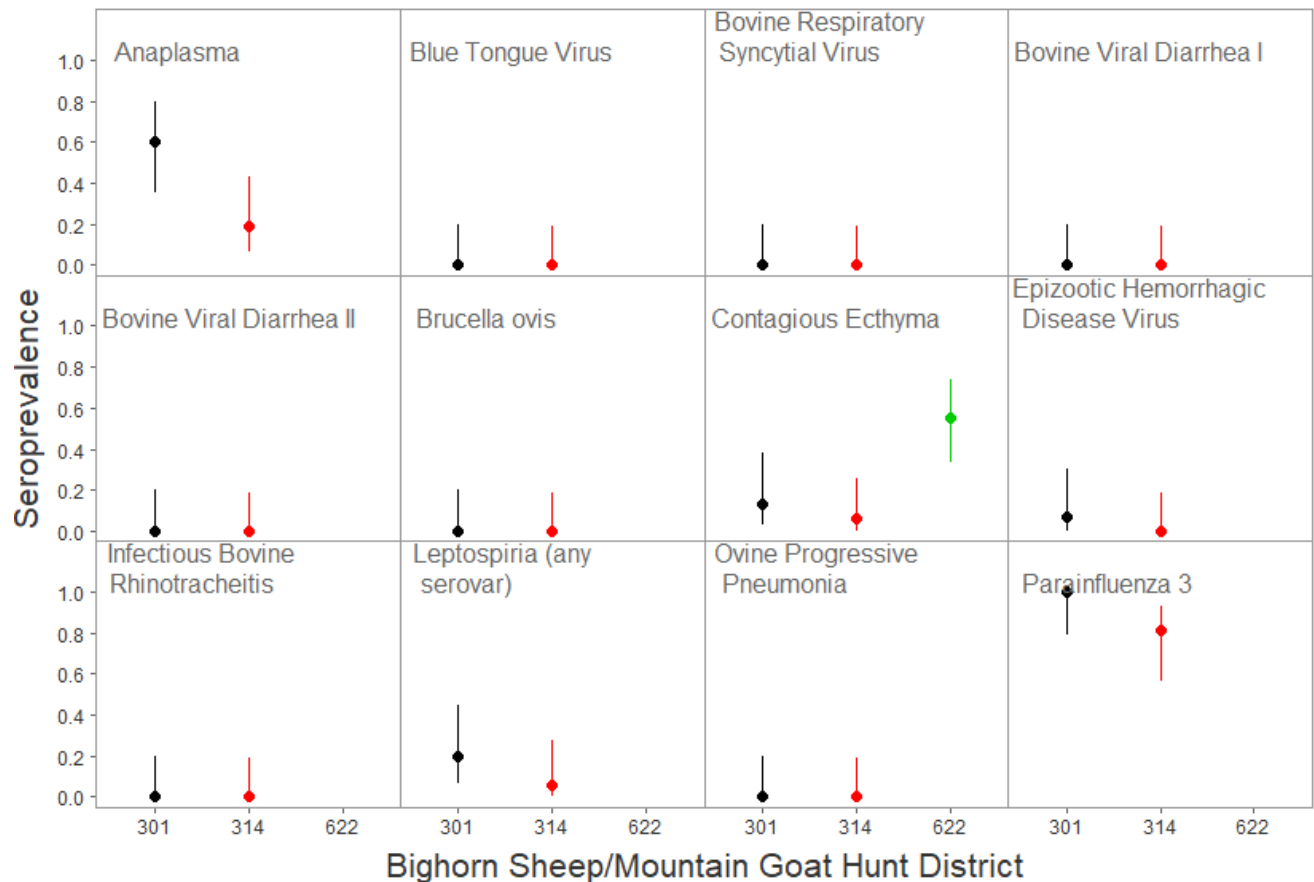


Figure 6. Summary of raw seroprevalence (proportion testing positive) and associated 95% binomial confidence intervals for pathogen exposures for moderate-risk pathogens, by herd. Because recent pathogen exposure data (2016 & 2017) existed for BHS from HD 622, we did not repeat the full panel of pathogen exposure and parasite assays for this herd (see Almberg et al. 2017). We did test for exposure to contagious ecthyma in HD 622 during 2018, which is displayed here.

Table 2. Summary of raw prevalence (proportion testing positive), 95% binomial confidence intervals (in brackets), and sample sizes for parasite infections by herd.

Pathogen	Test Type	Perceived Riskiness	HD 301 BHS	HD 314 MG	HD 622 BHS
<i>Psoroptes ovis</i>	Clinical	High	Not observed	Not observed	Not observed
Lungworm - <i>Protostrongylus</i> spp.	Baermann Fecal Float	Moderate	1, [0.76, 1], n=12	0.18, [0.05, 0.48], n=11	1, [0.72, 1], n=10
Lungworm - <i>Muellerius</i> spp.	Baermann Fecal Float	Moderate	0, [0, 0.24], n=12	0, [0, 0.26], n=11	0, [0, 0.28], n=10
Coccidia	Fecal Floatation	Low	1, [0.76, 1], n=12	1, [0.74, 1], n=11	0.7, [0.40, 0.89], n=10

Trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for sheep in HD 301 (Figure 7) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Serum-based selenium

concentrations in HD 301 fell below the published reference ranges for wild and domestic sheep.

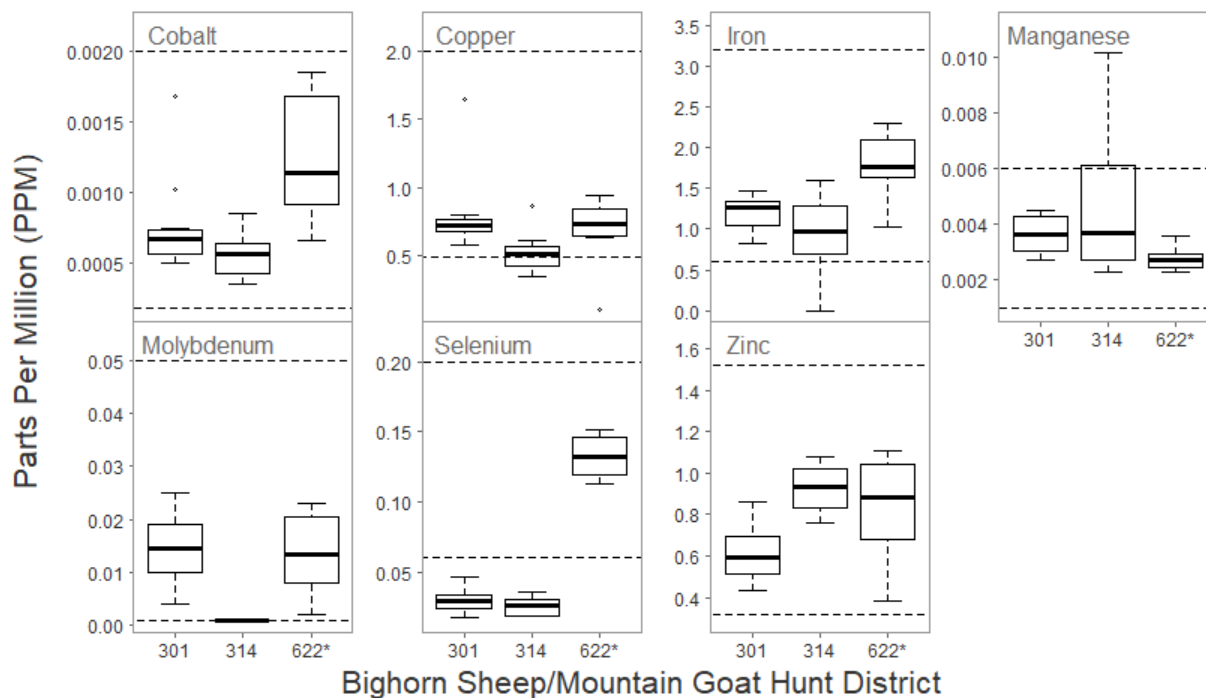


Figure 7. Boxplots of trace mineral concentrations in serum for cobalt, copper, iron, manganese, molybdenum, selenium, and zinc (displayed in parts per million, ppm) for each sampled bighorn sheep and mountain goat herd. Horizontal dashed lines represent the minimum and maximum range of values observed in wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Sample sizes were as follows: HD 301 (n=12), HD 314 (n=10), and HD 622 (n=8). *HD 622 was not sampled for trace minerals in 2018; values here are from health sampling conducted in 2016.

The Spanish Peaks herd had an average body condition score of 4.2 (95%CI: 3.9, 4.5) on a scale from 0.5-6 (Figure 8).

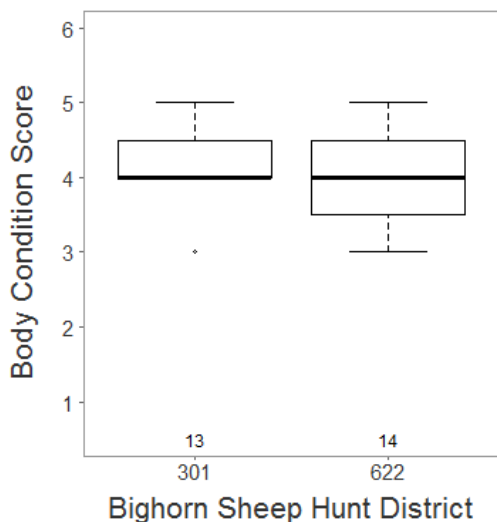


Figure 8. Boxplots of adult (≥2 yrs) female body condition scores (scale of 0.5-6, following a protocol developed by Tom Stephenson, personal communication) assessed by palpation for each sheep herd. Sample sizes are noted. This body condition scoring protocol has not been validated on mountain goats. A more coarse assessment of body condition is reported in text for the Gallatin Crest mountain goats.

Eight out of 9 sampled adult female bighorn sheep (89%, 95%CI: 57-98%) were pregnant based on the presence of Pregnancy-Specific Protein B in serum.

MG HD 314: Gallatin Crest Mountain Goats

On February 12, 2018, FWP and Quicksilver Air captured 19 mountain goats in the Gallatin Crest herd, including 9 adult females, 8 adult males, 1 yearling male, and 1 female kid. Within this herd, we found evidence of exposure to *Mycoplasma ovipneumoniae* with 29% (95%CI: 13-53%) of sampled goats testing seropositive. However, we failed to detect *Mycoplasma ovipneumoniae* by PCR (0%, 95%CI: 0-17%, power = 0.76) (Figure 4 & Figure 5). We did not detect any hemolytic *Pasteurellaceae*, but we lacked sufficient power to do so (Figure 5). We detected additional *Mannheimia ruminalis* and *Mannheimia* species, but none of these were hemolytic (Figure 4). We found that 13% (95%CI: 4-38%) of animals tested positive for the leukotoxin A gene from *Pasteurellaceae* cultured from tonsil swabs. In addition, we detected exposure to several moderate risk pathogens (Appendix I), including contagious ecthyma, anaplasma, *Leptospira* sp, and parainfluenza type-3, and detected active infections with *Protostrongylus* lungworm species (Figure 6; Table 2).

We are unaware of published trace mineral reference values for mountain goats. However, the concentrations of cobalt, iron, manganese, and zinc for goats in HD 314 (Figure 7) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herdt & Hoff 2011). Copper and molybdenum levels were towards the lower end of ranges reported for sheep. Selenium levels were lower than reference values for sheep, but within ranges previously reported for mountain goats (Samson et al. 1989).

While we do not have a standardized body scoring protocol for mountain goats, we did coarsely classify individuals as being in poor, fair, good, and excellent condition. Of 7 adult females scored, 3 were considered fair and 4 in good condition; of 8 adult males, 6 were considered fair, and 2 in good condition; the yearling male was in good condition, and the young of year female was in fair condition.

Ten out of 12 sampled adult female mountain goats (83%, 95%CI: 55-95%) were pregnant based on the presence of Pregnancy-Specific Protein B in serum.

BHS HD 622: Middle Missouri Breaks Bighorn Sheep

On February 10, 2018, FWP and Quicksilver Air captured 20 BHS in the Middle Missouri Breaks herd, including 14 adult females, 4 yearling females, and 2 yearling males, for translocation to the HD 122 Cut-off herd. We found no evidence by serology or PCR for exposure/infection with *Mycoplasma ovipneumoniae* (Serology: 0%, 95%CI: 0-16%; PCR: 0%, 95%CI: 0-16%, power of detection= 0.78) (Figure 4 & Figure 5). We detected the presence of hemolytic *Mannheimia haemolytica*/glucosida and *Mannheimia* sp./*Mannheimia ruminalis* and found that 50% (95%CI:

30-70%) of animals tested positive for the leukotoxin A gene on tonsil swabs. We did not detect *Pasteurella multocida* or hemolytic *Bibersteinia trehalosi*, but we lacked the power to do so. We detected additional *Mannheimia* species and *Bibersteinia trehalosi*, but none of these were hemolytic. Because recent pathogen exposure data (2016 & 2017, see Almberg et al. 2017) existed for this herd, we did not repeat the full panel of pathogen exposure and parasite assays. We did however, test for exposure to contagious ecthyma, for which we found serological evidence of recent exposure (55% seropositive, 95%CI: 34-74%).

Again, because of recent information on trace mineral concentrations for the Middle Missouri Breaks herd (Almberg et al. 2017), we did not repeat these assays. In recent years (2016-2017), trace mineral concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc for sheep in HD 622 (Figure 7) were within range of previously published reference values for wild (Poppenga et al. 2012) and/or domestic sheep (Herd et al. 2011).

The Middle Missouri Breaks herd had an average body condition score of 4 (95%CI: 3.6, 4.3) on a scale from 0.5-6 (Figure 8).

Females from Middle Missouri Breaks herd were not assessed for pregnancy status in 2018.

Discussion

In February 2018, we conducted health assessments on two BHS herds, including the Spanish Peaks (HD 301) and Middle Missouri Breaks (HD 622) herds, and one MG herd, the Gallatin Crest (HD 314) herd. Both the Spanish Peaks BHS and Gallatin Crest MGs were evaluated to help inform decisions regarding their management and use as potential source herds for future translocations. The Middle Missouri Breaks BHS received health assessments in 2016 and 2017 and was sampled again in 2018 when 20 sheep were captured and translocated into Region 1's Cut-off herd (HD 122). All three herds exhibited some evidence of infection with at least one pathogen of concern should they be mixed with naïve sheep or goats (Figures 5, 6, & 7). Both the Spanish Peaks BHS and Gallatin Crest MGs exhibited evidence of infection with *Mycoplasma ovipneumoniae*. By contrast, after three years of sampling, the Middle Missouri Breaks BHS remain apparently free from infection with *Mycoplasma ovipneumoniae*. We detected the Leukotoxin A gene in all three herds, as well as hemolytic *Mannheimia haemolytica*/*glucosida* in the Spanish Peaks and Middle Missouri Breaks herds. While we failed to detect additional hemolytic *Pasteurellaceae*, our statistical power for detection was too low (<0.80; Figure 5) to confidently declare their absence from these three herds. In addition, all three herds exhibited evidence of infection with contagious ecthyma, which when introduced to naïve sheep/goats, can cause debilitating sores to form around the mouth, in some cases leading to death.

Trace element concentrations in each sampled herd appeared adequate according to previously published reference ranges in wild and domestic sheep, except for selenium, for which concentrations were low among Spanish Peaks BHS and Gallatin Crest MGs. Concentrations of copper and molybdenum among the Gallatin Crest MGs were towards the lower end of ranges reported for sheep, but, without published reference ranges, these may be normal values for

goats. Adult female body condition indices, measured in February, were similar across both sampled BHS herds. Because animals were sampled in February when lactation status is less reliably assessed, we were unable to summarize body condition in relation to lactation status, despite being known as an important predictor of body condition in other species elsewhere (Cook et al. 2013, Proffitt et al. 2016). Both the Spanish Peaks BHS and the Gallatin Crest MG pregnancy rates were within the range of published pregnancy or reproductive rates for wild BHS (70-93%; Brundige et al. 1988, Berger 1991) and MG (~70-90%; Hamel et al. 2010, Festa-Bianchet et al. 1993) populations.

Respiratory disease has been identified as one of the largest and most damaging health issues facing BHS. There is increasing scientific evidence that *Mycoplasma ovipneumoniae* is a necessary agent involved in pneumonia outbreaks (Besser et al. 2012, Besser et al. 2013, Cassirer et al. 2017, Butler et al. 2018). However, research has revealed substantial variation in morbidity, mortality, recruitment rates and herd performance in the presence of *Mycoplasma ovipneumoniae*, suggesting that other factors, including strain type or co-infecting agents, may be influential. Among 14 *Mycoplasma ovipneumoniae*-positive herds in Montana (n=7) and in Wyoming (n=7) (Butler 2017), at least half of the herds have exhibited adequate lamb recruitment and adult survival, and have recently been stable or increasing, despite some history of pneumonia epizootics. Conversely, we are unaware of populations that have a recent or more distant history of pneumonia epizootics in which *Mycoplasma ovipneumoniae* has not been detected, since diagnostic tools for *Mycoplasma ovipneumoniae* have become available. Therefore, based on the evidence accumulated to date, *Mycoplasma ovipneumoniae* is likely a necessary agent involved in pneumonia epizootics, but may not be a sufficient indicator of a history, severity, or likelihood of pneumonia epizootics. We hope that with future sampling across additional herds, we will have more information with which to evaluate the role of *Mycoplasma ovipneumoniae* and other co-infecting agents in wild sheep and goat respiratory disease.

Both the Spanish Peaks BHS and Gallatin Crest MGs appear to be infected with *Mycoplasma ovipneumoniae*. While neither herd has had a documented all-age pneumonia die-off, we have detected individual pneumonia-related deaths in both herds. Despite this, both herds are currently stable or growing. In 2016, the Gallatin Crest MG herd was estimated to be the third largest mountain goat herd in the state (population estimate = 250 as compared to a median size of 49 across 54 herds; Smith & DeCesare 2017) and the Spanish Peaks BHS herd the sixth largest bighorn sheep herd in the state (population estimate = 170 as compared to a median size of 59 across 29 herds; unpublished MFWP data).

We failed to detect *Mycoplasma ovipneumoniae* in the Middle Missouri Breaks herd, both on serology and PCR, for the third year in a row. Serological tests typically indicate past exposure and are generally recognized as the more sensitive test to assess recent or current infection at the herd level (i.e., an animal doesn't have to be actively shedding the pathogen to be detected as being or having been infected). These two pieces of information combined suggests that *Mycoplasma ovipneumoniae* remains absent from the Middle Missouri Breaks. The Middle Missouri Breaks is among the state's most robust BHS herds, and its size coupled with its

negative *Mycoplasma ovipneumoniae* status contributed to FWP's decision to use it as a source population for a translocation to HD 122's Cut-off herd in 2018.

Pasteurellaceae, particularly hemolytic strains or those that contain (and express) the Leukotoxin A gene, are thought to play important roles in respiratory disease, influencing patterns of morbidity and mortality (Dassanayake et al. 2010, Besser et al. 2012, Shanthalingam et al. 2014, Wood et al. 2016). Recent work has indicated that detection probabilities are extremely low (0.12-0.36) for the majority of *Pasteurellaceae* using culture-based diagnostic methods (Butler 2017, Butler et al. 2017, Walsh et al. 2012). Our sampling effort attempted to accommodate these low detection probabilities by testing multiple tonsil swabs per animal by culture. However, because we did not meet target sample sizes (30), we still faced low statistical power to detect most of these pathogens (Figure 5). We did, however, detect the Leukotoxin A gene by PCR in all three sampled herds. Interestingly, the detection of the leukotoxin gene is not always associated with hemolytic activity of the bacteria (the ability of the bacteria to lyse red blood cells), which is assumed to be the sign of toxin expression (Fisher et al. 1999). This indicates that the gene is present in the *Pasteurellaceae* bacteria within these herds, but that perhaps it was not being expressed; it is unknown how this relates to future risk of gene expression and associated hemolytic activity.

Management Implications

Given that the Spanish Peaks BHS and Gallatin Crest MGs are infected with high-risk respiratory pathogens, especially *Mycoplasma ovipneumoniae* and *Pasteurellaceae* with the Leukotoxin A gene, where possible, we would recommend against using these herds as source populations for future translocations. These two herds are also positive for contagious ecthyma, and we would advise against translocating sheep or goats positive for contagious ecthyma into a naïve herd. Although these two herds appear demographically robust, experience elsewhere suggests that moving animals potentially infected with high-risk respiratory pathogens into a naïve herd could trigger epidemics.

The Spanish Peak BHS and Gallatin Crest MGs are interesting, although not unique, in that they remain stable and are among the state's largest herds, despite hosting some of the key respiratory pathogens like *Mycoplasma ovipneumoniae*. The Spanish Peaks herd has experienced pneumonia-related mortalities and years of poor lamb recruitment, both of which are consistent with the effects of respiratory disease. Although these herds are currently among the state's largest BHS and MG herds, they are small relative to estimates of historic herd sizes. Most of Montana's BHS herds are infected with respiratory pathogens (Appendix 2, Figure A2.2), which raises the possibility that we have few 'disease-free' herds against which to compare the demographic rates of infected herds.

The BHS translocated from the Middle Missouri Breaks herd (HD 622) to the Cut-off Herd (HD121) appears to have been successful. Nineteen of the 20 sheep remain alive (one was almost immediately killed after release by a mountain lion). During a survey flight in April, 2018, biologist Bruce Sterling observed 60 sheep in the Cut-off herd, including 11 of 19 collared translocated animals.

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Appendix I

All captured mountain goats and bighorn sheep will be screened for the following list of pathogens and parasites. This table also includes an assessment of the risk associated with each pathogen/parasite and an associated rationale, as well as the test types and laboratories employed for the screening. Testing laboratory abbreviations: WADDL=Washington Animal Disease Diagnostic Laboratory; FWP =Montana Fish, Wildlife, and Parks Health Laboratory; DOL=Montana Department of Livestock Laboratory; MSU DCPAH=Michigan State University Diagnostic Center for Population and Animal Health.

Organism	Risk category	Rationale	Test type and any limitations	Testing Laboratory
<i>Mycoplasma ovipneumoniae</i>	High	Likely necessary, if not sufficient, for chronic or epidemic BHS pneumonia.	Serology—gives us most conservative metric of presence/absence from a herd. PCR—the proportion of actively infected individuals may be small and chronically infected individuals may shed intermittently, so a “negative” at the herd level may not give us as much confidence.	WADDL
Leukotoxin A+ <i>Pasteurellas</i>	High	Identified as a potentially important class of pathogens involved in respiratory disease.	PCR	WADDL
Hemolytic <i>Pasteurellas</i>	High	Identified as a potentially important class of pathogens involved in respiratory disease.	Culture	WADDL
<i>Mannheimia haemolytica</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Bibersteinia trehalosi</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Pasteurella multocida</i>	Moderate	Sometimes identified in respiratory disease; exact role unknown.	Culture/PCR	WADDL
<i>Psoroptes ovis</i>	High	Highly contagious and capable of causing chronic and extensive morbidity and mortality.	Parasitology	FWP/DOL
Lungworm	Low	Depending on burden; low burdens are normal, high burdens may be problematic.	Parasitology	MSU DCPAH
Contagious ecthyma (Orf)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
<i>Brucella abortus</i>	Low	Not generally considered a problem for sheep/goats.	Serology	DOL

<i>Brucella ovis</i>	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
<i>Anaplasma</i>	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine respiratory syncytial virus (BRSV)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine herpesvirus (IBR)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Parainfluenza-3 virus (PI3)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Ovine progressive pneumonia (OPP)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bovine viral diarrhea (BVD I & II)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Bluetongue virus (BTV)	Moderate	Would not want to mix infected with naïve individuals.	Serology	DOL
Epizootic hemorrhagic disease (EHD)	Low		Serology	DOL
<i>Leptospira</i> (CAN, ICT, HAR, GRIP, POM serovars)	Low	Environmentally transmitted and widely distributed. Probably not a major concern.	Serology	DOL

APPENDIX II: Supplementary figures and tables

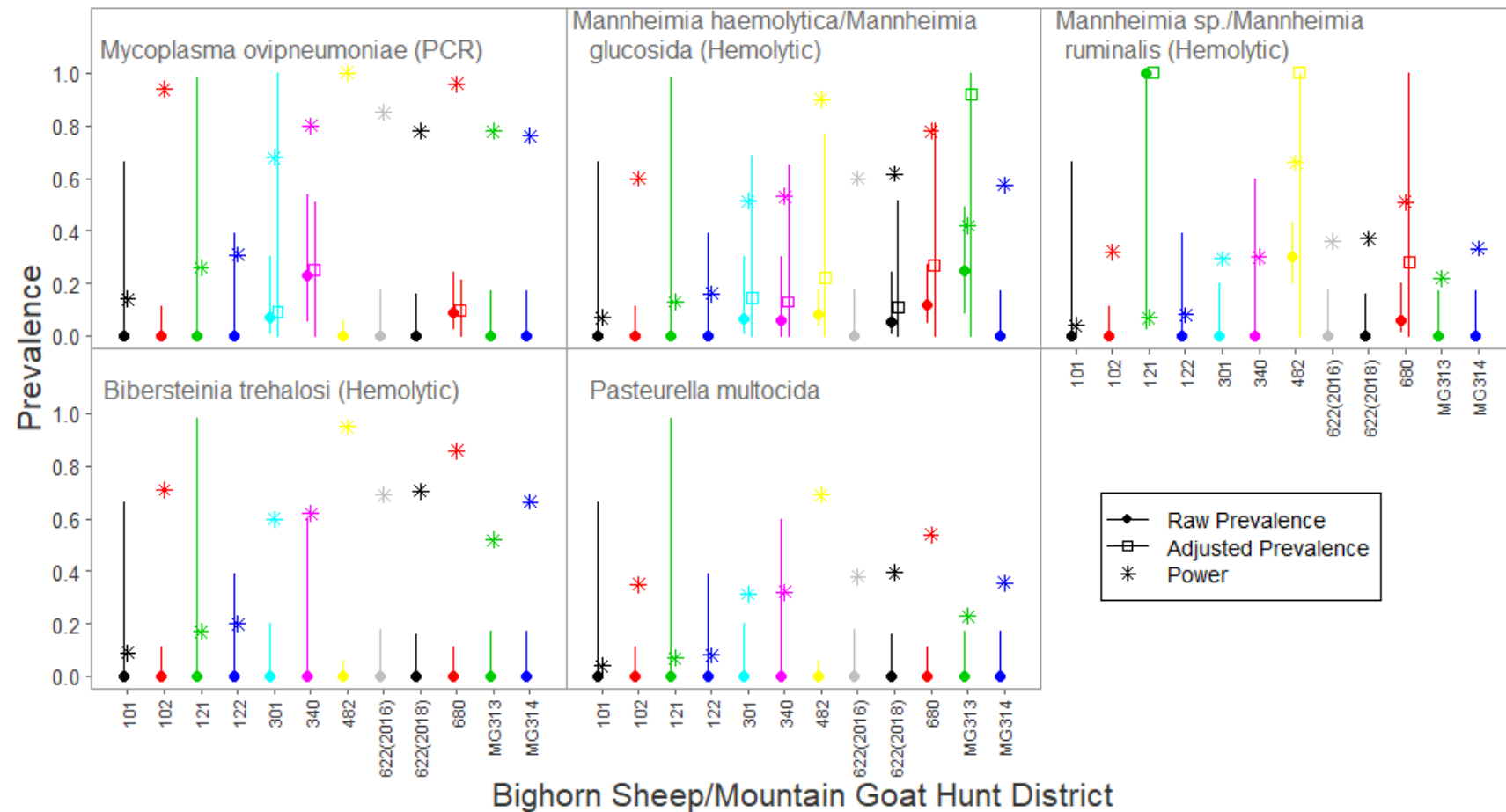


Figure A2.1. Summary of estimated raw and adjusted (for imperfect detection) prevalences, and associated 95% confidence intervals, as well as estimated power to detect select respiratory pathogens in bighorn sheep and mountain goat herds sampled during 2016 - 2018 for P-R grant W-166-SI to Montana Fish, Wildlife & Parks. Adjusted prevalences were calculated by dividing the raw estimates by estimated detection probabilities from Butler (2017), and corrected 95% confidence intervals were calculated using the delta method. Adjusted prevalences were only calculated when raw prevalence was >0. We estimated the statistical power to detect the pathogen if it were present at 10% prevalence given the herd size, our sample size, number of swabs collected per animal, and the detection probability using the

approach detailed in Butler (2017). Point estimates and 95% confidence intervals for raw prevalences were estimated using the binomial distribution. All data is from bighorn sheep, except that from MG313 and MG314, which are from mountain goats caught in the Crazy Mountains and on the Gallatin Crest.

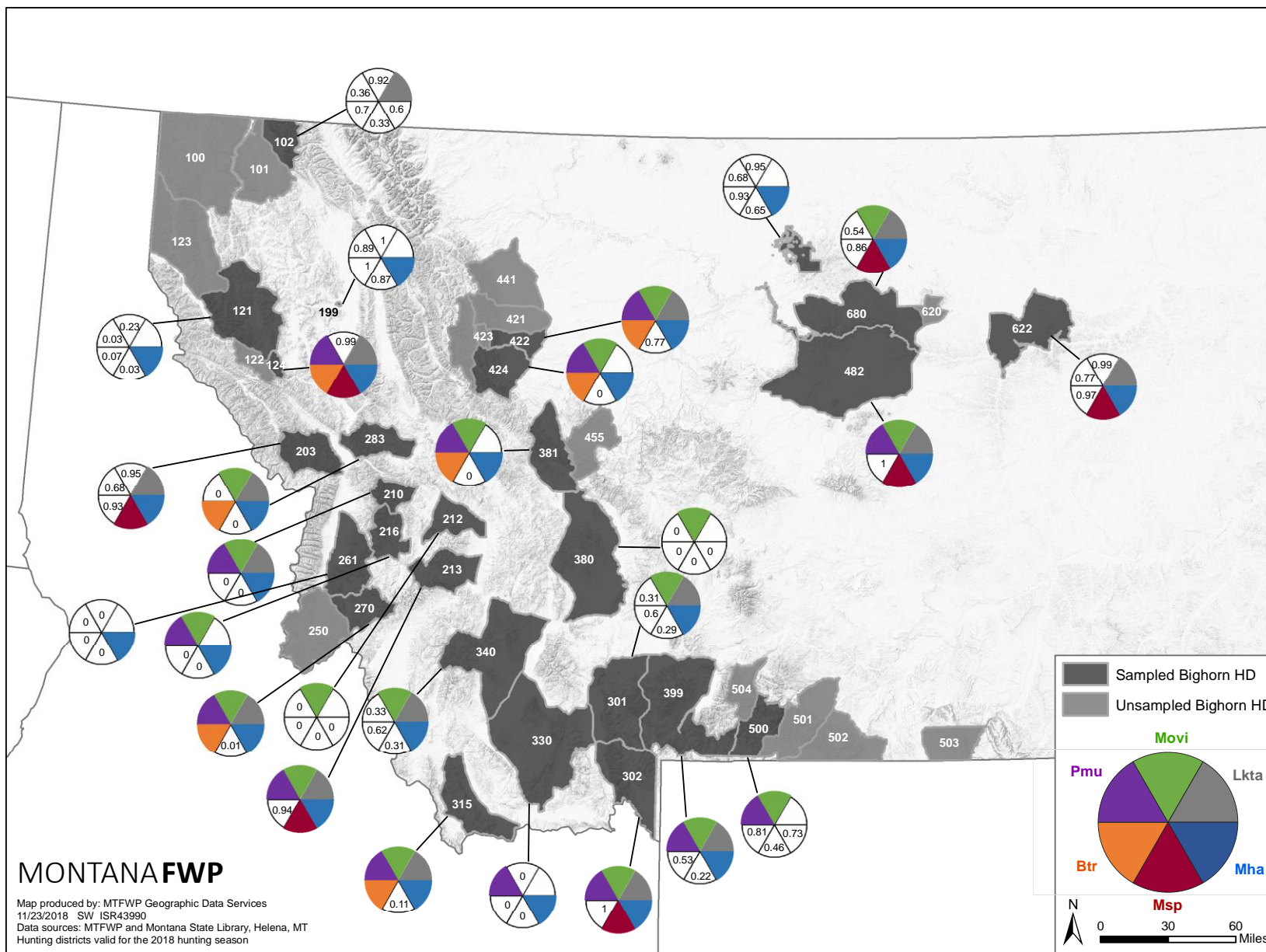


Figure A2.2. Map displaying bighorn sheep herds sampled for respiratory pathogens. Data comes from state-wide live-animal captures, hunter harvest, or mortalities submitted to the Wildlife Health Lab from 1991-2018. Pathogens are as follows: Movi = *Mycoplasma ovipneumoniae*, detected by PCR; Lkta = Pasteurella Leukotoxin A gene, detected by PCR; Mha = hemolytic *Mannheimia haemolytica* or *Mannheimia glucosida*, detected by culture; Msp = hemolytic *Mannheimia species* or *Mannheimia ruminalis*, detected by culture; Btr = hemolytic *Bibersteinia trehalosi*, detected by culture; Pmu = *Pasteurella multocida*, detected by culture. Any detection from a bighorn sheep on nasal, tonsil or lung swabs from within the hunt district between 1991-2018 is reported. In cases where there have been no detections, the numbers displayed within empty pieces of the pie chart represent our power to detect the pathogen if it were present at 10% prevalence, given sample size, number of swabs collected per animal, and the detection probability using the approach detailed in Butler (2017). Power calculations only included data collected between 2011-2018 from live animal captures (nasal or tonsil swabs). There is no estimate for detection probability of the Leukotoxin A gene, so no power is reported. Figure is based on version created by Butler et al. 2018.